REMARKS

Status of the Claims

Claims 1-44 are in the application.

Claims 1-40 were rejected.

Claim 41-44 have been withdrawn from consideration.

Claims 1, 21, 22, 26, 27, 28, 34, 37 and 40 were objected to.

By way of this amendment claims 1, 5, 20-22, 26-28, 34, 37 and 40 have been amended and claims 41-44 have been canceled.

Upon entry of this amendment, claims 1-40 will be pending.

Summary of the Amendment

Claims 1, 20-22, 26-28, 34 37, 38 and 40 have been amended to replace the numeral that refer to steps or elements in the claims with letters or lower case roman numerals.

Claim 1 has also been amended to more clearly set forth subject matter.

Step c has been amended to clarify that molecules are among the cDNA that are produced when both the cDNA are cDNA is reannealed from the mixture of denatured first and second populations of cDNA.

Since the first population of cDNA molecules and the second population of cDNA molecules were tagged using different tags, and the cross-hybridized double stranded cDNA molecules contain one strand from the first population of cDNA molecules and one strand from the second population of cDNA molecules, each of the cross-hybridized double stranded cDNA molecules contains both tags. Step d has been amended to more expressly state that the cross-hybridized double stranded cDNA molecules that contain both tags are isolated.

The cross-hybridized double stranded cDNA molecules that have one strand from each of the two populations of cDNA molecules, may not have exactly complementary sequences. Such mismatches represent differences between the otherwise complementary sequences derived from

two different populations of cDNA and exist on the cross-hybridized double stranded cDNA molecules as single stranded nucleic acid, i.e. without complement. Step e is amended to more expressly set forth that from the cross-hybridized double stranded cDNA molecules contain both tags which were isolated in step d, the cross-hybridized double stranded cDNA molecules that contain both tags and that have mismatched sequences which are present as single stranded cDNA are selected.

Step f has been amended to more clearly set forth the step of coupling the two strands of the cross-hybridized double stranded cDNA molecules that contain both tags and that have mismatched sequences which are present as single stranded cDNA which were selected in step e. As amended, the claim expressly recites that the 5' end of one of the strands is coupled to the 3' end of the other strand which essentially forms a single nucleic acid molecule that is self hybridized in a hairpin fashion. When, denatured, the arms of the hairpin release from each other and a single nucleic acid molecule exists as a linear single stranded nucleic acid molecule.

Step g has been added to include the step of actually denaturing the hairpin single nucleic acid molecule produced by coupling in step f to form a linear single stranded nucleic acid molecule.

The linear single stranded nucleic acid molecule formed from coupling the two strands of the mismatch-containing, cross-hybridized, double stranded cDNA molecules and then denaturing the coupled cross-hybridized double stranded cDNA molecules comprise a region that is the strand of the first population of cDNA molecules, the region corresponding to the coupler and a region that is the strand of the second population of cDNA molecules. By comparing the sequence corresponding to the region from the first population of cDNA molecules with the region from the second population of cDNA molecules the sequences corresponding to the mismatch represent the location of alternative splices of cDNA from the first population of cDNA and the second population of cDNA.

Claim 5 has been amended to correct an obvious error. As amended, claim 5 is clearly dependent on claim 1 and contains the limitation that the first population of cDNA and the second population of cDNA are derived from different species.

Claim 20 has also been amended to more clearly disclose the nature of the two different selectable tags used to tag and distinguish the first population of cDNA and the second population of cDNA.

Support for the amendments is found throughout the specification. No new matter has been added.

Claim Objections

Claims 1, 21, 22, 26, 27, 28, 34, 37 and 40 are objected to because of the use of numerical designations for the method steps. Claims 1, 21, 22, 26, 27, 28, 34, 37 and 40, and claim 20 have been amended to replace all numerical designations with letters or lower case Roman numerals. In view of the amendment, the bases for objection are removed. Applicant respectfully requests the objection to claims 1, 21, 22, 26, 27, 28, 34, 37 and 40 be withdrawn.

Claim Rejections – 35 U.S.C. § 112

Claims 1-40 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

It is asserted that claims 1-40 are indefinite and confusing because it is unclear in steps (5) and steps (6) of claim 1 how one is to select for regions of single-stranded nucleic acids when no prior steps allude to the presence of any single stranded nucleic acid acids in the mixed population.

Step e (former step 5) has been amended to state that cross hybridized cDNA that "comprises at least one area of mismatched sequence present as an unhybridized single-stranded nucleic acid" is selected from the cross hybridized cDNA,and that this isolated in step d (former step 4). In view of the amendment, it is clear that the single-stranded nucleic acid is an area of mismatch in the cross hybridized cDNA.

It is also asserted that the claims are indefinite because it is "unclear as to how the "coupling" step or any of the prior steps results in determining alternative spliced RNA molecules from normally spliced counterpart RNA molecule." It is asserted that claim 1 contains "a gap between the actual method steps and the final step of detecting alternative spliced RNA molecules from the normal spliced counterpart". It is asserted that none of the steps provide "a clear nexus to detection of any alternative spliced sequences." As amended, the steps of claim 1 clearly define the method of identifying alternative spliced cDNA from two populations of cDNA. Step a refers to obtaining two populations of cDNA. Step b refers to tagging each of the two populations of cDNA the tags used are different to that one population of cDNA is tagged with one type of tag and the other population of cDNA is tagged with a different type of tag. Step c refers to forming cross hybridized cDNA which contains one strand from one population of cDNA and the other strand from the other population of cDNA. It is noted that the strand that make up the cross hybridized cDNA have different tags such that the cross hybridized cDNA has both tags. Step d refers to isolating cDNA that has both tags, i.e. the cross hybridized cDNA, formed in step c. Step e refers to selecting from the isolated cDNA, those cDNA which include a mismatch, i.e. non-complementary sequences which are present

as single stranded DNA. Step f has been amended to more clearly set forth that the two strands of the cDNA selected in step e are coupled at one end by linking the 5' end of one strand to the 3' end of the other. Doing so results in formation of a hairpin which if denatured yields a single stranded DNA molecule rather than two single stranded cDNAs. Newly added step g refers to the denaturation of the coupled, i.e. hairpin double stranded cDNA to form a single stranded DNA molecule. Step h has been amended to more clearly set forth that a comparison the sequence of the region derived from one strand with the sequence of the region derived from the other strand reveals the mismatched sequences which correspond to the alternative splices. As amended , claim 1 clearly and definitely sets forth each step to identify alternative splice forms.

Claim 5 has been rejected as being indefinite and confusing because it cannot be determined if Applicant intends for the claim to be an independent claim or if Applicant intends for the claim to be a dependent claim. Claim 5 has been amended to correct an obvious error. As amended, claim 5 is clear and definite.

Claim 20 has been rejected as being indefinite and confusing because it is asserted that a clear interpretation of Applicant's intent cannot be ascertained. It is asserted that it cannot be determined the actual structure of the tag required for the instant invention. Claim 20 has been amended to make it more clear and definite. As amended, claim 20 refers to a pair of oligonucleotides in each of element i) and ii), and further sets forth how one strand of the pair is different from the other. As amended, claim 20 is clear and definite.

As amended, claim 1-40 are clear and definite. Applicant respectfully requests that the rejection of claims 1-40 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention be withdrawn.

Claim Rejections – 35 U.S.C. § 103

Claims 1-19 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Schweighoffer et al. (US 6251590, June 2001) in view of Winkler et al (20040110191, January 2001).

Schweighoffer et al. teach a method of identifying alternatively (differentially) spliced nucleic acid regions occurring between two physiological conditions, comprising cross-hybridization between RNA and cDNA populations belonging to distinct physiological states thereby allowing for the identification of unpaired regions, i.e., regions present in RNA in one physiological conditions and not in RNAs from another physiological conditions. Schweighoffer et al. teach that such regions essentially correspond to alternative forms of splicing, typical of a given physiological state. Schweighoffer et al. do not expressly teach the use of tags to assist in the detection of alternatively spliced sequences belonging to distinct physiological conditions.

Winkler et al. teach a method comprising obtaining a first population of a target molecule from a biological sample and a second population of target molecule from a biological sample. Winker et al. teach attaching a first selectable tag to cDNA molecules of the first cDNA population and a second, different selectable tag to cDNA molecules of the second cDNA population, forming a

mixed population of cDNA and performing amplification of the mixed population of cDNA molecules to compare the relative abundance of the target molecules in different populations.

It is asserted that one of ordinary skill in the art at the time of the claimed invention would have been motivated to include unique selectable tags as taught by Winkler et al. in the cross hybridization method of Schweighoffer et al. to improve the means of detecting target nucleic acid species present in the cDNA populations in a single reaction vessel. It is asserted that one of ordinary skill in the art at the time of the claimed invention would have been motivated to use selectable tags for detecting alternatively spliced sequences in a mixed population because the use of distinguishable tags are within the scope of the ordinary artisan's capabilities because their use is not incompatible with the ability to detect variations within multiple nucleic acid sequences.

It is asserted that Schweighoffer et al. teach using biological samples from normal or diseased tissue, or tissues or cells corresponding to different physiological conditions, thereby meeting the additional limitations allegedly set forth in claims 2-9.

It is asserted that Winkler et al. teach RNA population comprising polyA+ RNA for detection by tag which thereby renders claim 10 obvious in view of the combination of Schweighoffer et al. and Winkler et al.

Likewise, it is asserted that Winkler et al. teach that the cDNA population comprises double stranded cDNA which thereby renders claim 11 and 12 obvious in view of the combination of Schweighoffer et al. and Winkler et al, and that Winkler et al. teach the tag may comprise a labeling domain, a restriction enzyme domain, a secondary amplification domain, a secondary differentiation

domain, or a sequencing primer binding domain hereby renders claim 13-19 obvious in view of the combination of Schweighoffer et al. and Winkler et al.

Applicants respectfully disagree and urge that the combination of Schweighoffer et al. and Winkler et al. does not render the present invention obvious. The combination of references does not yield the claimed invention. Neither Schweighoffer et al. nor Winkler et al. teach or suggest coupling the two strands of the cross hybridized cDNA to form a single nucleic acid molecule that includes sequences from each source including the mismatched sequences. This feature set forth in step f (former step 6) is neither taught nor suggested in either reference. It is well settled that in order to establish a prima facie case of obvious, the combination of references must teach or suggest the claimed invention. The combination of Schweighoffer et al. and Winkler et al. does not teach or suggest a method which includes the step of coupling the two strands of a double stranded DNA molecule to form a single stranded nucleic acid molecule with both strands of the mismatched pair. The combination of Schweighoffer et al. and Winkler et al. does not render the claims 1-19 obvious.

Moreover, Schweighoffer et al. approached to problem of identifying splice variants through a different approach. No modification of Schweighoffer et al using the teachings of Winkler et al. would yield the claim method. No modification of Schweighoffer et al would yield the claim method.

For the foregoing reasons, Applicants respectfully urge that claim 1-19 are not obvious over the combination of Schweighoffer et al. and Winkler et al. Applicants respectfully request that the rejection of claims 1-19 under 35 U.S.C. § 103(a) as being unpatentable over Schweighoffer et al. in view of Winkler et al be withdrawn.

Conclusion

Claims 1-40 are in condition for allowance. A notice of allowance is earnestly solicited. Applicants invite the Examiner to contact the undersigned at 610.640.7855 to clarify any unresolved issues raised by this response.

The Commissioner is hereby authorized to charge any deficiencies of fees and credit of any overpayments to Deposit Account No. 50-0436.

Respectfully submitted,

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